Characterization of Resistance to *Pratylenchus thornei* (Nematoda) in Wheat (*Triticum aestivum*): Attraction, Penetration, Motility, and Reproduction

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ABSTRACT

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Lines from a cross between two wheat (*Triticum aestivum*) cultivars with contrasting resistance phenotypes to *Pratylenchus thornei* (Nematoda) were investigated to determine the stage at which resistance occurs. Host resistance was examined at nematode attraction to and penetration of roots and nematode motility, maturation, and reproduction within roots. There was no significant difference in the rate at which *P. thornei* was attracted toward or penetrated resistant or susceptible roots. However, suppression of migration, juvenile maturation, and reproduction in and near resistant roots was evident, suggesting that resistance acts post

penetration. No preferential root penetration zone was observed in contrast to other studies. The inhibitory compounds from resistant wheat plants appeared to be constitutively expressed and water soluble because nematode migration was suppressed in roots and root exudates of unchallenged seedlings. The effects of these compounds were reversible and affected *P. thornei* but not *P. neglectus*. Apart from migration, nematode multiplication was greatly inhibited by resistance because only a few juveniles (10%) developed past stage three in roots of resistant compared with susceptible plants. Earlier in the life cycle, egg deposition and hatch of *P. thornei* were also significantly reduced in resistant roots and root exudates, suggesting the presence of hatching inhibitors.

Additional keywords: molting, root lesion nematode.

Several *Pratylenchus* spp. occur in Australia on various hosts, with two dominant in cereals, *Pratylenchus thornei* and *P. neglectus* (61). Necrotic lesions form as a result of cell death caused by *Pratylenchus* nematodes feeding on root cortical cells (37,79,80). Cortical degradation and reduced root branching diminishes the plant's ability to acquire nutrients and water from the soil, stressing the plant and reducing vegetative growth and yield. The value of wheat production lost in Australia due to *P. thornei* is estimated at >\$AUD 50 million each year (39).

The process by which *Pratylenchus* spp. invade and develop can be separated into four main components: root recognition, penetration, feeding and migration, and reproduction. Initially, the nematodes are attracted to the root and probe the surface for an acceptable penetration site. After a salivation period, the nematode may feed ectoparasitically on root hairs. Using stylet thrusting and body movement, they penetrate the root epidermis (79), enter the root, and migrate intracellularly while feeding endoparasitically on root cortex cells (80). During migration, at each new cell, the nematode punctures the cell wall with stylet thrusts and pushes through into the adjacent cells to continue feeding (32,79,80). While in the roots, adult *Pratylenchus* nematodes deposit eggs in the cavities created by migration (2). Eggs are also deposited in the soil in close vicinity to the roots (11,40). The juvenile hatches from the egg as a stage-two juvenile (J2) and, at each molt, undergoes further sexual development (52). The

full cycle from egg to adult is completed within 45 to 65 days (67) and is greatly influenced by host, temperature, and *Pratylenchus* sp.

Due to the wide host range of *Pratylenchus* spp. and the restrictions, costs, and inefficiency of chemical nematicides, the development of resistant cultivars has become increasingly important as a control measure (1,59). Most studies of wheat–*Pratylenchus* interactions have focused on the genetic inheritance of resistance (61,59). The identification of new genetic sources of *P. thornei* resistance and the identification of resistance quantitative trait loci (QTL) and associated molecular markers is critical, due to the lack of resistance available in current commercial cultivars. However, knowledge of the biological or biochemical resistance mechanisms is also important because it can provide insights into the nature, timing, and action of resistance genes which could provide new sources of biochemical control and reveal the involvement of different resistance genes, which could be pyramided for a more durable resistance (27). In addition, the understanding of resistance at the biological and biochemical level could be used to improve phenotypic screening methods. Common phenotypic resistance screening techniques are laborious, time consuming, and inherently variable because they involve extracting and counting nematodes from the roots. DNA-based detection methods can achieve higher throughput (42) but are expensive. The identification of a resistance mechanism at a specific stage of invasion or within a particular tissue may enhance screening procedures by reducing these associated costs and time. However, there are gaps in the knowledge of the biochemistry and molecular biology of plant defense responses to migratory endoparasitic nematodes, largely because they cause widespread cellular destruction and lack a fixed feeding site.

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Only a small number of studies have considered the effects of resistance on *Pratylenchus* biology. Talavera and Vanstone (58) demonstrated that *P. thornei* is able to penetrate resistant cultivars and Farsi (14) observed equal root penetration of *P. neglectus* in both resistant and susceptible wheat lines. Other studies in various plant hosts have shown that, in other *Pratylenchus* spp., resistance is associated with reduced motility and reproduction (3,45,48,64). It is hypothesized that resistance in wheat to *P. thornei* occurs post root penetration and causes reduced reproduction by either the suppression of migration through the cortex which, in turn, suppresses feeding; the suppression of egg deposition and hatching; or inhibition of nematode maturation (Fig. 1).

In this study, the parents of a cross between two wheat cultivars with contrasting resistance phenotypes to *P. thornei*, 'Sokoll' (resistant) and 'Krichauff' (susceptible), in addition to six selected lines from the population were investigated. The chosen population lines were selected based on their resistant genotypes as determined by QTL analysis (Linsell, Rahman, Taylor, Davey, Gogel, Wallwork, Forrest, Hayden, Taylor, Oldach, *unpublished data*). The first aim was to compare the early stages of the invasion process of *P. thornei*, including root attraction, penetration, and nematode motility within the root and in root exudates (REs), in these resistant and susceptible genotypes in order to identify the stages where and how resistance has an effect. The second aim was to determine whether the resistance within these genotypes is due to reduced juvenile development, egg deposition, and egg hatch. The investigation aimed to generate useful screening protocols, gain a better understanding of the biological resistance mechanisms, and provide a foundation for directed biochemical investigations into *P. thornei*-resistant wheat responses.

MATERIALS AND METHODS

Nematodes. *P. thornei* was obtained from wheat at Nunjikom-pita, South Australia and cultures were maintained on carrot callus, as described by Moody et al. (38). Cultures were stored at 22°C and subcultured every 3 months. To collect the nematodes,

carrot callus was placed in funnels in a misting chamber with an intermittent aqueous mist of 10 s every 10 min for 96 h (56). Nematodes extracted in the mister were counted in 250- μ l aliquots in three replicates and diluted with water to the required inoculum concentration. Because *P. thornei* is a parthenogenic species, most of the nematodes collected were females. To obtain inoculum of nematodes of a single developmental stage, suspensions were passed repeatedly through sieves. Adults and stage-four juveniles (J4s) were captured on a 40- μ m sieve, stage-three juveniles (J3s) on a 30- μ m sieve, and J2s were retained on a 20- μ m sieve. Eggs but few nematodes were able to pass through the 20- μ m sieve. This sieving method provided relatively high purity; nevertheless, the presence of nonrequired stages was recorded.

Plant material. The study investigated a doubled-haploid (DH) population from a cross between the synthetic-wheat derived Sokoll (*P. thornei* resistant) and the Australian wheat Krichauff (*P. thornei* susceptible). This population was phenotyped using DNA quantification methods and genetically mapped to identify two highly significant resistant QTL on chromosomes 2B and 6D (Linsell, Rahman, Taylor, Davey, Gogel, Wallwork, Forrest, Hayden, Taylor, Oldach, *unpublished data*). The parents and six selected individual lines (three susceptible [S1, S2, and S3] and three resistant [R1, R2, and R3]) were analyzed. The six lines were selected based on genotypic and phenotypic data. The three resistant lines had both resistant QTL and the three susceptible lines had neither resistance QTL. The chosen resistant and susceptible selected lines contained the lowest and highest amounts of quantified *P. thornei* DNA, respectively. For all experiments, prior to germination, seed were surface sterilized by submersion in 70% ethanol for 5 min, then hypochlorite (15%) for 20 min at room temperature (RT) of 21 to 22°C, followed by four rinses with reverse osmosis (RO) water.

Nematode inoculation and extrapolation. For experiments on agar, 3-day-old wheat seedlings with a primary leaf and a 20-mm seminal root were placed on 0.5% water agar in 90-mm petri plates at RT. After 48 h, plates were inoculated with *P. thornei* by pipetting suspensions onto the surface. Nematodes on the agar were counted using a stereomicroscope (M3C; Wild, Heerbrugg,

Fig. 1. Life cycle of *Pratylenchus thornei* associated with *Triticum aestivum*, with special reference to host resistance characterization stages.

Switzerland) and a grid template (10-mm block segments). To count nematodes that had entered roots, seedlings were placed in a misting chamber for 96 h and were extracted and counted. Nematode suspension (≈ 10 ml) was collected and stored at 4°C until counted in 500- μ l aliquots using a stereomicroscope, and the total number in the root system was estimated accordingly. Alternatively, nematodes that could no longer be seen on the agar surface were assumed to have penetrated the roots (initial inoculum number – number of nematodes remaining on agar plate = number of nematodes within roots). This was confirmed in a check experiment at 12 h after inoculation (hai), where the total number of nematodes extracted from the agar via misting plus the total number that had entered the roots equaled the initial inoculum number.

For experiments in pots, plants were grown in a glasshouse maintained at $20 \pm 3^\circ\text{C}$. One DH seed was sown in steam-pasteurized sand in each seedling tube (55 by 120 mm) in a five-by-five randomized block arrangement. Seven days after planting, each seedling was inoculated with *P. thornei* by pipetting two 500- μ l aliquots of nematode suspension into two 50-mm-deep holes on either side of the seedling. Inoculum densities were specific for each experiment and are described later. At 2 days after inoculation (dai), slow-release fertilizer (Osmocote; Scotts Miracle-Gro) was added (4 g/kg of sand) to the tubes. Plants were hand watered every 2 days to soil water holding capacity. Nematodes were extracted by placing the washed root systems into a misting chamber, as described above.

Attraction. Movement of *P. thornei* in response to the presence of roots of susceptible and resistant genotypes was investigated in sand and on agar systems. For all experiments on agar, it was assumed that nematodes that could no longer be seen had penetrated roots and were expressed as a percentage of the initial inoculation number.

Attraction on agar. Five replicates of 5-day-old seedlings of both parents were placed on 0.5% water agar. Plates were divided into 10-mm segments, with roots occupying 30 mm at one end. In the first 20 mm at the opposite end to the roots, inoculum of 150 mixed-stage *P. thornei* was added (Fig. 2A). A no-root control plate was included with the same plate design but excluding active seedling roots. The number of *P. thornei* on each plate was counted. At 6, 9, 12, 27, 30, 33, 36, and 48 hai, the number of nematodes in each segment was counted and their location on the plate grid recorded, reflecting the distance nematodes had moved toward the roots over time. The assay was repeated twice.

Attraction on agar across REs. Six seedlings for both Sokoll and Krichauff were grown aseptically in 14-ml glass vials on nylon mesh in 8 ml of sterile RO water. RE was collected after roots had been submerged in water for 7 days. Ten independent replications were collected and the experiment was repeated

twice. A known susceptible seedling ('Machete') was placed at one end of the 0.5% water agar plate to stimulate nematode movement. RE was pipetted onto the plate in front of the root system so that, in order to reach the roots, the nematodes had to move across the RE solution. The same gridded plate described above (Attraction on agar) was used but 1 ml of RE was placed in a 20-mm segment 30 mm from the plate edge (Fig. 2B). Plates were inoculated with 500 mixed-stage *P. thornei* 30 mm from the RE and 30 mm from the plate edge so that nematodes could either move toward or away from the exudates. After 48 h, the number and location of individual nematodes on the plates was assessed. The numbers of nematodes that remained at the point of inoculation, which moved away from the root (repelled), and moved toward the root (attracted) were expressed as a percentage of the initial inoculation number.

Attraction with both genotypes present. Sixteen different resistant versus susceptible combinations made from the parents and the six DH lines were assessed either in sand or on agar. These same combinations were twice analyzed independently both in sand and on agar. For experiments in sand, one resistant and one susceptible seedling was sown into the same pot (55 by 120 mm) and inoculated with two 500- μ l aliquots (500 nematodes/pot) into two 50-mm-deep holes between the two seedlings. Roots were harvested after 14 days, freed from sand, and placed on the mister for nematode extraction. For experiments on agar, resistant and susceptible 5-day-old seedlings were placed at either end of a 90-mm plate with 0.5% water agar. The plate was inoculated with 225 mixed-stage nematodes in a 30-by-10-mm rectangle near its center (Fig. 2C). After 9 days, roots were placed on a mister for nematode extraction and collection. Attraction was assessed by measuring the percent penetration of the resistant and susceptible root in each combination. Then, the number of combinations that had more penetration in the resistant or susceptible roots was calculated and presented as a percentage of the total number of combinations analyzed.

Penetration. Rates of penetration in sand and on agar. The rate of *P. thornei* penetration was assessed both in sand and on agar systems. For experiments in pots, seedlings were grown in sand under glasshouse conditions ($20 \pm 3^\circ\text{C}$) and were inoculated with 1,500 mixed-stage *P. thornei* nematodes/plant. Roots were harvested at 5, 7, 10, and 16 dai, washed, and placed on the mister. The parents and two lines from each of the resistant and susceptible lines were assessed with five replicates. For agar analysis, 3-day-old seedlings that had been germinated on moistened sterile filter paper were placed at one end of a plate (0.5% water agar) and inoculated with 150 mixed-stage nematodes/plant at the opposite end. Three independent analyses were conducted using three replicates of the parents and three lines of both resistant and susceptible genotypes. Nematodes visible on the plate were

Fig. 2. *Pratylenchus thornei* attraction and migration assay plate designs. Plates divided into 10-mm segments with seedlings occupying the first 30 mm. **A**, Attraction on agar: nematodes placed in distal 20 mm (gray shaded). **B**, Attraction on agar across root exudates: root exudates in 30-mm space (dark gray shaded) and nematodes in 20-mm space (light gray shaded) 30 mm from root exudates and edge of plate. **C**, Attraction with both genotypes present: resistant and susceptible seedlings at either end of plate with nematodes placed in center 30-mm space (gray shaded). **D**, *P. thornei* migration: using strips of plastic, all of the root system was restricted so that only a 250-mm-long section of one root was exposed for nematode penetration. Nematodes were inoculated near the tip of the exposed root.

counted at 6, 9, 12, 30, and 36 hai. The number of nematodes that had penetrated roots was calculated (initial inoculum number – number visible on plate at time point) and means were expressed as a percentage of the initial inoculation number.

Penetration of adults versus juveniles on agar. Differences in rates of penetration of adults and juveniles was assessed in sand and agar systems. For experiments in sand, seedlings were inoculated with 500 adult nematodes/plant or 500 J2 nematodes/plant. Roots were harvested at 3, 4, 6, and 8 dai. Two resistant and susceptible lines and both parents were assessed with five replications. For experiments on agar, seedlings on 0.5% water agar were inoculated with 1,000 nematodes/plant and the numbers of visible nematodes were counted at 6, 12, 24, and 30 hai. The number of *P. thornei* that penetrated roots was calculated and means at each time point were expressed as a percentage of the initial inoculation number.

Impact of crushed root suspensions and REs on motility. The effect of resistance on *P. thornei* motility was investigated with in vitro assays in REs and crushed root (CR) suspensions. A nematode was considered nonmotile if no movement was observed within 5 s. The concentration of RE and CR suspensions were standardized to minimize variation between roots, because larger root systems are expected to produce more REs and contain more root compounds when crushed than smaller roots. Thus, all RE or suspension samples were adjusted to a set optimum concentration of 4 mg/ml of fresh weight root tissue in water, as determined by testing motility suppression in an RE dilution series.

REs. Seedlings were grown aseptically in petri plates on sterile filter paper for 10 days at RT. Three replicates of each of the six DH lines and the parents were analyzed. RO water (1 ml) was used to wash the roots, and filter paper and the liquid was collected. Fresh roots were weighed and the collected RE adjusted to a concentration of 4 mg/ml of fresh weight root tissue by addition of RO water. A 100- μ l aliquot of RE was transferred to individual wells (350 μ l) on a 96-well enzyme-linked immunosorbent assay (ELISA) plate and inoculated with $\approx$ 80 mixed-stage nematodes. Three replicates of each sample were assessed. Numbers of motile and nonmotile nematodes were counted at 1, 2, and 3 dai at RT. These assays were performed on both *P. thornei* and *P. neglectus*.

To determine whether the effect of resistant RE on *P. thornei* motility could be reversed, nematodes were recovered from the resistant Sokoll (10 samples) RE after 1 day of exposure into water, and motility was reassessed after 2 days.

CR suspensions. One seed was sown in each seedling tube (55 by 120 mm) in steam-pasteurized sterilized sand. Three replicates of each of the four DH lines (two resistant and two susceptible) and the parents were analyzed. Plants were grown for 1 week in a glasshouse maintained at $20 \pm 3^\circ\text{C}$ and were hand watered every 2 days with 100 ml. Plants were then washed and fresh root weights were recorded. Roots were crushed in liquid nitrogen and immediately suspended in RO water. The CR suspensions were centrifuged and supernatant removed and adjusted to a concentration of 4 mg/ml of fresh weight root tissue by addition of RO water. Experiments were conducted either in 6 ml of CR suspension in sample containers (40 by 45 mm) or 100 μ l of CR suspension in wells on an ELISA plate. Each replicate was inoculated with $\approx$ 80 mixed-stage nematodes. Numbers of motile and nonmotile nematodes were counted using a stereomicroscope (M3C; Wild) at 3 and 7 dai at RT.

Migration. The effect of resistance on *P. thornei* migration within the root cortex was investigated with a root exclusion assay on agar. Seedlings were germinated on sterile filter paper and then placed on 0.5% water agar for 3 to 4 days at RT. Three replicates of each of the six DH lines and the parent sample were analyzed. Using strips of plastic, all of the root system was blocked off except a 250-mm section of one root. Water agar (1%) was then

used to cover the rest of the roots and the plastic strips acted as a wall so that only the 250-mm root portion was exposed for nematode penetration (Fig. 2D). In all, $\approx$ 100 mixed-stage nematodes were inoculated near the exposed root. At 5 dai, the seedling was removed from the plate and the roots were stained in acid fuchsin (7). Stained roots were placed in petri plates and, using a microscope, the inoculation point and the distance nematodes had migrated from that point on the exposed root was marked on the plate and measured.

Reproduction and maturation. *P. thornei* development within resistant and susceptible roots was assessed in sand over 45 days (one generation). Five replicates of the parents and six DH lines (three susceptible and three resistant) were grown in glasshouse conditions, as described above. Each plant was inoculated with 500 J2s. Roots were harvested at 5, 10, 15, 24, 30, 36, and 45 dai and nematodes were extracted by misting. These times were chosen based on the timeline observed by Larson (33). The number of J2, J3, and J4 or adult *P. thornei* present at each of these times was counted and means at each time point were calculated. Developmental stages were assessed by size after separation through sieving and counted with a dissecting microscope. The J4s and adults were counted as one group. The maturation data set had a non-normal distribution and was transformed using natural logs.

Egg deposition. The effect of resistance on *P. thornei* egg deposition was investigated by observing mature females in the presence of roots on agar. Five replicates of the parents and three DH resistant and susceptible lines were assessed. Seven-day-old seedlings on water agar (0.5%) were inoculated with 1,000 J4 and adult *P. thornei* nematodes adjacent to the roots. No eggs were present. After 10 days at RT, the numbers of eggs on the agar were counted under a stereomicroscope. The roots were then stained in acid fuchsin, and eggs deposited by adults that had entered the roots were counted.

Hatching. The rate of *P. thornei* juvenile hatching was assessed by exposing mature eggs to roots grown on agar and to RE with the same set of lines as for egg deposition in five replicates.

For analysis on agar, seed were germinated on moist filter paper and 6-day-old seedlings were transferred to water agar (0.5%) media plates and inoculated with 80 *P. thornei* eggs directly at the roots. At RT 8 dai, the hatched juveniles on the agar were counted. To count juveniles that had migrated into the roots, juveniles were extracted by misting (misting chamber) or roots were stained with acid fuchsin.

For analysis in RE, seedlings were grown aseptically in petri plates on sterile filter paper for 12 days at RT. REs were taken from young plants because *P. thornei* hatching was shown to be greater in RE from younger plants (47). RO water (1 ml) was used to wash the roots and filter paper and the liquid was then collected. Fresh roots were weighed and collected exudates were adjusted to 4 mg/ml of root tissue. The RE (100 μ l) was transferred to individual wells on a 96-well ELISA plate. In all, $\approx$ 80 eggs were added to each well (350 μ l) and the numbers of hatched J2s were counted at 3, 7, and 10 dai. Hatching assays were also performed in dilutions of 4, 10, and 100 mg/ml of root tissue to determine the presence of hatching inhibitors (HIs). Five replicates of the parents and three DH resistant and susceptible lines were assessed twice. Because *Pratylenchus* hatching is influenced by temperature, experiments were performed at the optimum temperature of 21 to 22°C (10).

Statistical analysis. Replicate means were calculated and were pooled for each sample from each independent assay to give one data set for each experiment. Normally distributed data sets were analyzed by analysis of variance (ANOVA) with Fisher's least significant difference post-hoc test to compare means, where $P < 0.05$ was considered statistically significant using the statistical program Genstat (VSN International). Correlation coefficients were calculated and used to interpret trends. Prior to ANOVA

analysis, data sets were checked for normality and homogeneity of variances, and were log-transformed where necessary to correct deviations from these assumptions.

RESULTS

Attraction. *Attraction on agar.* The rate at which *P. thornei* moved across the agar toward the roots over 36 h was not significantly ($P = 0.05$) different between genotypes. At each of the seven intervals assessed (6, 9, 12, 27, 30, 33, and 36 hai), there were equal numbers of *P. thornei* in each grid (data not shown). After 36 hai, the total number that had reached and entered the root was not significantly ($P = 0.05$) different.

Attraction on agar across RE. This assay measured nematode movement in response to resistant and susceptible RE. To reach the root system of the susceptible Machete (which acted to stimulate nematode movement), nematodes had to move through RE. Eight Sokoll, seven Krichauff, and six no-root control replications were analyzed. At 48 hai, there was no statistically significant difference between nematode movement away or toward the RE within or between genotypes (Table 1). Generally, 23% (115 nematodes) were attracted to the roots and $\approx 15\%$ (78 nematodes) were repelled. The majority, with 65% or 325 nematodes, remained at the point of inoculation. In the no-root control where there was no RE barrier, a similar number moved away and toward the roots.

TABLE 1. Percent *Pratylenchus thornei* movement on agar in response to root exudates from the resistant (Sokoll) and susceptible (Krichauff) *Triticum aestivum* genotypes 48 h after inoculation

Genotype	<i>n</i>	Attracted (%)	Repelled (%)	Stationary (%)
Sokoll	8	22.6	16.6	70.7
Krichauff	7	23.3	14.3	60.8
No-root control	6	15.1	14.2	62.4
Standard error	...	9.2	7.7	7.6

TABLE 2. Attraction of *Pratylenchus thornei* (measured as penetration) to 16 different resistant and susceptible *Triticum aestivum* combinations assessed in sand (14 days after inoculation) and on agar (9 days after inoculation), where roots of both genotypes were available for nematodes to penetrate

Penetration (%)	Resistant	Susceptible	<i>n</i>
Sand	52.2	47.8	13
Standard error	9.1	5.2	...
Agar	16.7	81.3	14
Standard error	4.4	4.4	...

Attraction with both genotypes present. Across two repeated experiments, 16 different resistant and susceptible combinations, including the parents and DH lines (S1, S2, S3, and Krichauff versus R1, R2, R3, and Sokoll) were assessed in sand and agar for their ability to attract *P. thornei*. Some replications were excluded due to poor plant growth, leaving data from 13 combinations in sand and 14 on agar to be analyzed. When both resistant and susceptible roots were available for penetration on agar, significantly ($P = 0.05$) more *P. thornei* were attracted to the susceptible roots (80%) 9 dai; however, in sand, attraction to both genotypes 14 dai (50%) was the same (Table 2). On average, in 11 of the 14 different resistant and susceptible root combinations tested, more *P. thornei* were attracted toward and penetrated the susceptible roots and, in three combinations, more *P. thornei* entered the resistant roots.

Penetration. *Rates of penetration in sand and on agar.* The penetration on agar was much quicker and was measured in hours whereas days were necessary for the sand system. There was no relationship between the rate of *P. thornei* penetration and genotype on agar or in sand when measured 36 hai and 16 dai, respectively. However, there was a significant increase in penetration over time in both genotypes (Fig. 3). Significantly ($P = 0.05$) higher penetration occurred in the agar system than in sand, with $>50\%$ penetration occurring after only 9 to 10 hai, whereas penetration in the sand system never reached 50%.

Penetration of adults versus juveniles on agar. There was no difference in the rates of J2 or adult penetration between genotypes. However, comparison of adult and juvenile penetration showed a significant relationship between developmental stage and penetration rate, with a correlation coefficient of 0.4 in both media. On agar, the rate of juvenile penetration was higher than adults but, in sand, it was reversed (Fig. 4). At 30 hai on agar, J2 penetration was almost 40% more than adult penetration. In comparison, at 8 dai in sand, adult penetration was 30% higher than J2, with only 7% total J2 penetration.

Root penetration zones. *P. thornei* nematodes were observed probing the root surface in various root zones but were most frequently detected at the junction of lateral branches and seminal main root axes (Fig. 5A to D). In addition, large groups were often observed both in the zone of elongation (Fig. 5E) and in seminal roots near the hypocotyl. *P. thornei* nematodes were seen within the dense root hair regions of both seminal main axes (Fig. 5F) and lateral branches but penetration in this zone was not observed. Once the nematodes had penetrated the root, large groups of *P. thornei* at various developmental stages, including eggs, were often seen aggregated in different regions of the root (Fig. 5G).

Fig. 3. *Triticum aestivum* root penetration of *Pratylenchus thornei* on agar and in sand over A, 36 h and B, 16 days, respectively, in resistant (Sokoll, R1, and R2) and susceptible (Krichauff, S1, and S2) genotypes. LSD = least significant difference.

Motility and migration. The motility suppression of RE at 2, 4, and 100 mg/ml was assessed and the 4 mg/ml concentration was found to produce the best differentiation between the resistant and susceptible genotypes (Table 3) and, thus, was used in all motility experiments.

There was greater *P. thornei* motility suppression in both resistant RE and CR suspensions after exposure for 3 days than in suspensions of susceptible genotypes (Fig. 6A and B). A small amount of motility suppression occurred in Krichauff and the susceptible lines (30%) but was equal to the no-root control. The resistant Sokoll RE and CR suspensions caused significant loss of motility at 65 and 75%, respectively. In order to determine the specificity of the observed motility suppression, assays were also conducted on the closely related species *P. neglectus*. There was no effect on *P. neglectus* motility when exposed to RE for 3 days because motility suppression was equivalent to the no-root control (25%) (Fig. 7). The effect of resistant root compounds appeared partially reversible in Sokoll RE because recovery in water, after 2 days of exposure, resulted in almost 50% of *P. thornei* that had been immotile regaining motility. In the resistant Sokoll RE after 1 day of exposure, motility suppression was 94%. The ability of *P. thornei* to migrate within resistant roots was also suppressed (Fig. 8). Migration within the root was significantly ($P = 0.05$) lower in resistant root genotypes (Sokoll, R1, R2, and R3) than in susceptible (Krichauff, S1, S2, and S3). On average, the migration of *P. thornei* from a set point of penetration was 10 mm in Sokoll compared with 68 mm in Krichauff.

Reproduction and maturation. Nematode development in the susceptible lines showed the molt of J2 to J3 taking 7 to 10 days and subsequent molts to J4 and adult occurring within 18 to 28 days. Egg deposition occurred within 2 days after observing mature females, and J2 hatched 7 to 10 days after.

When comparing the developmental time frames between resistant and susceptible genotypes at each developmental stage, there were significantly ($P = 0.05$) higher numbers of *P. thornei* in the susceptible Krichauff parent than the resistant Sokoll (Fig. 9). Nematode numbers were log transformed [$\ln(x + 1)$] and are hereafter referred to as log units followed by (e^{x-1}). There was no second-generation hatch of J2 at 36 dai (Fig. 9A) in Sokoll or any of the resistant lines but, in Krichauff, a mean of 1.24 (13.3) juveniles and, on average, 3.4 (30) juveniles resulted in the susceptible lines. The inoculated J2s molted to J3 ≈ 10 dai but no significant difference in numbers occurred between genotypes (Fig. 9B). However, at 45 dai, the numbers of J3s in the second generation was significantly ($P = 0.05$) higher in Krichauff at 4.08 (52.3) and the susceptible lines C01 and C35 than in Sokoll at 0.9

(5.3) and the three resistant lines. The J3s molted to J4 and adult at ≈ 15 to 26 dai. The number of J4s and adults measured at 24 dai (Fig. 9C) was 10 times higher in Krichauff at 2.58 (44.7) than in Sokoll at 0.64 (4.7). Thus, juvenile development was suppressed as early as the molt from J2 to J3.

Egg deposition and egg hatch. The number of eggs deposited by adults placed directly adjacent to roots of seedlings on agar after 10 days was statistically higher in the presence of susceptible genotypes than resistant. The mean number of eggs deposited in or near Krichauff was 53 compared with 18 in Sokoll, giving an egg deposition rate of 4.7 and 1.5 eggs/day, respectively (Fig. 10A and B). Significantly ($P = 0.05$) more eggs were deposited within susceptible roots (83%) than outside (17%) whereas 50% of eggs were laid both within and outside the resistant roots. Only 4 eggs were laid in the absence of roots.

Resistant genotypes also stimulated significantly ($P = 0.05$) less hatching than susceptible genotypes. After 10 days, the mean number of juveniles hatched from eggs placed adjacent to resistant Sokoll and susceptible Krichauff roots was 4 and 48, respectively, giving respective hatching rates of 0.5 and 5 juveniles/day. There was no statistically significant difference in the position of juveniles (within or outside roots) after 10 days between genotypes (Fig. 11A). There were high hatching rates (45%) in the no-root control, similar to that of Krichauff. Exposure of eggs to RE suppressed hatching in the resistant genotypes, with 17% hatch in Sokoll and 25% in Krichauff after 3 days (Fig. 11B). In the no-root water control, only 8% hatch occurred. The dilution of RE corresponded to an increase in hatching in both genotypes. There was statistically significant greater egg hatch at a concentration of 4 mg/ml compared with 100 mg/ml in all genotypes (Fig. 12).

DISCUSSION

Root attraction and penetration. Recognition of and attraction to host roots are essential steps that precede nematode root invasion. Host recognition is thought to involve signals from the root that can stimulate egg hatch or attraction toward the roots (44,46). It is generally accepted that nematodes locate roots through chemotactic factors diffused from the host (78). It has been suggested that signal elicitors from the host are water soluble so that they can move through soil toward the nematode (45). REs are regarded as the most probable stimuli in attracting *Pratylenchus* spp. to the root region (5,70,71). However, gradients in temperature, electrochemical potential, pH, and carbon dioxide also play a role (46). Specific nematode repellents or attractants

Fig. 4. *Triticum aestivum* root penetration of second-stage juvenile (J2) or adult developmental stages of *Pratylenchus thornei* A, in sand and B, on agar over 8 days and 36 h, respectively. LSD = least significant difference.

have not been identified but may include amino acids, sugars, phenolics, and other secondary metabolites (18).

REs contain a mix of positive and negative effectors which can attract, repel, or be neutral in terms of nematode behavior (49,76,77). Thus, the effects of REs depend on the combination of signals at a specific time (19,68,73). In this study, there was no difference in the rate (both number and speed) at which *P. thornei* was attracted toward (23%) or away (15%) from resistant or susceptible roots when only one genotype was present. In addition, in the sand media, when both resistant and susceptible roots were available for penetration, no preference was observed for *P. thornei* attraction after 9 days. This suggests that wheat REs have a neutral effect on *P. thornei* attraction. Other studies, which utilized similar agar and sand biological systems, have also observed that resistance does not interfere with nematode root attraction. For example, *Meloidogyne incognita* migration to resistant and susceptible cotton roots in sand and *M. naasi* juveniles attraction to resistant and susceptible wheat and barley roots on agar were equal in both genotypes (4,36).

Contrasting observations have been made, however, and preferential attraction has been observed to susceptible roots when both resistant and susceptible genotypes are present in other nematode-plant interactions when analyzed in soil. Bias toward the susceptible roots (72%) was observed in *M. hapla* when juveniles hatched between the resistant and susceptible lucerne (alfalfa) roots grown in soil. However, when only one root was present, there was equal attraction and penetration (20). Collectively, these results show that, in some interactions, plants can influence nematode movement toward roots in positive and negative ways. Preferential attraction was also observed in this study but only when attraction was investigated on agar. When both resistant and susceptible roots were present, significantly higher *P. thornei* attraction (>81%) occurred toward the susceptible roots 24 hai. The bias toward susceptible roots suggests that, when given a choice of both resistant and susceptible roots, either resistant root secretions deter *P. thornei* because they secrete repellent or toxic compounds early during prepenetration, as suggested by Berge (6), or the susceptible roots secrete compounds making them more attractive. The susceptible roots might not produce the repellent compound or produce it in lower concentrations than the resistant roots. When penetration on agar was measured again at 36 hai, there was no difference between resistant and susceptible roots, implying that *P. thornei* may be able to tolerate the effects of resistant root chemicals by activating a protective mechanism. Similar to the observations here, in a resistant potato cultivar, few *M. fallax* juveniles penetrated the roots 24 hai but, at 60 hai, penetration numbers were comparable with the susceptible cultivar (31). This suggests that the active compounds in resistant root secretions may only influence nematode movement for a limited period, possibly by a potential adaptation of the nematode.

The attraction bias to susceptible roots observed only in agar systems in the presence of both resistant and susceptible genotypes may be due to general differences between the experimental systems in sand (pots) and agar. Due to a greater concentration of active compounds in the small agar volume (35 ml) compared with the larger pot volume (6,600 ml), and with the agar system only being possible with young seedlings, a clear difference in the plants' age exists between agar and sand systems.

Because plant-feeding nematodes possess stylets and generally secrete cell-wall-degrading enzymes (51), they commonly successfully penetrate the host regardless of whether further development occurs. Thus, most nematodes seem capable of overcoming the physical protection barriers of plants. Resistance to root knot, cereal cyst, and root lesion nematodes investigated in several crop species has never been associated with inability to penetrate roots. Juvenile *M. incognita* in cotton and lucerne and several *Heterodera* spp. in wheat, soybean, and potato were able to successfully penetrate and migrate to the feeding site in both susceptible and

resistant cultivars (12,30,74). In addition, similar penetration rates were observed in susceptible and resistant bean cultivars when inoculated with *P. scribneri* 24 hai. Thus, it was hypothesized that penetration suppression plays no role in resistance of wheat to *Pratylenchus* spp. Accordingly, in this study, juvenile *P. thornei* penetration rates did not significantly differ for resistant and susceptible roots both on agar and in sand, even after 16 days. This is supported by Talavera and Vanstone (58), who observed *P. thornei* penetrating resistant wheat cultivars, and Farsi (14), who found no difference in penetration rates of *P. neglectus* between resistant and susceptible wheat cultivars. Neither attraction to roots nor penetration in sand was negatively affected by resistant genotypes; therefore, resistance to *P. thornei* in the resistant lines investigated here seems to act post penetration.

Penetration of developmental stages. All *Pratylenchus* developmental stages are able to penetrate and invade roots. However, the rate of penetration by adults in this investigation was significantly higher than that of J2 penetration in sand. Comparable observations were made in Lucerne, where *P. penetrans* adult females were more invasive than juveniles because they penetrated roots earlier, faster, and over a wider range of soil temperatures (62). The greater ability of adults to penetrate the strong cell walls is attributed to the size of the glands in the posterior subventral lobe, a more developed stylet, and a larger-sized body (one-third larger) compared with a J2 (24,75).

In contrast to the observations in sand, penetration on agar was significantly higher for J2s than adults, which can be explained by the differences in the three-dimensional structures of the substrates sand and agar. The higher penetration rates on agar may be due to their increased ability to move across this medium. Nematode movement in soil has been shown to be directly dependent on the size of soil pores (69). Zunke (80) observed that, due to longer ectoparasitic feeding on root hairs than adults, *P. penetrans* juveniles penetrated the root later, and Zunke suggested that extended root hair feeding was essential for juveniles to obtain nutrients for further colonization. Because juveniles on agar can more easily migrate toward the root, perhaps less ectoparasitic feeding is required.

Root penetration zones. There are differences in the sites and mechanisms of root penetration by different species of *Pratylenchus* in various hosts. In lucerne and clover, *P. penetrans* preferred to penetrate the main root axes, where lateral root branches ruptured the cortex (63). These nematodes then migrated through the cortex of main root axes into other lateral root branches. Other studies suggested that most *P. penetrans* aggregated and penetrated in the zone of root elongation (65,79). In lucerne, strawberry, and maize roots, the preferred zone of penetration was the dense root hairs (62). Zunke (80) observed *P. penetrans* penetrating and feeding on root hairs of various hosts, including rape, tobacco, and potato, but other studies indicated that *P. penetrans* only explored and penetrated the root hairs but did not feed on them (32,41). However, Castillo et al. (11) suggested that *P. thornei* penetrated the roots of chickpea without any site preference. In the current study, *P. thornei* were commonly observed at the junction where lateral roots branched from the main seminal root. However, large groups were often also observed both in the zone of elongation and in seminal roots near the hypocotyl. Although penetration was not observed, *P. thornei* were seen within the dense root hair regions of both seminal and lateral roots. *P. thornei* may feed on root hairs and then move elsewhere along the root to penetrate. In this study, groups of *P. thornei* at various developmental stages, including eggs, aggregated in various root regions. These observations suggest that *P. thornei* does not have a preferred site of penetration in wheat.

Motility and migration. When toxic or unfavorable conditions arise, many organisms respond by becoming inactive by arresting development (45) or by becoming so damaged that they lose the

ability to move. In this study, significantly higher numbers of *P. thornei* became nonmotile when exposed to REs (65%) and CR suspensions (75%) from resistant than susceptible (30%) roots. Similarly, several other studies have reported suppressed motility in resistant roots and associated this response with accumulation of phytoalexins. For example, in response to invasion by *M. incognita* and *Heterodera glycines*, soybean synthesized the isoflavonoid glyceollin in the stele of resistant cultivars within 3 dai, which inhibited nematode respiration and motility (23,28, 29). Increased production of isoflavonoids in response to *Pratylenchus* invasion was also observed in several plant species. Coumestrol inhibited *P. scribneri* motility in resistant lima beans by 50% after exposure for 96 h (48). Similarly, high levels of medicarpin and coumestrol were found in resistant lucerne root

tissue after invasion with *P. penetrans*, and motility was inhibited on agar by medicarpin (3). Despite the common link between isoflavonoids and *Pratylenchus* motility inhibition, in order to confirm the role of phytoalexins in providing resistance in the specific interaction between *P. thornei* and the resistance present in the Sokoll $\times$ Krichauff population, further work is required to identify specific compounds.

In both the RE and CR motility assays, some loss of nematode motility (20 to 30%) occurred in the no-root water control, similar to that of the susceptible samples because, in general, motility is never 100% in nematode populations. The suppression of motility in the resistant lines was greater in the CR suspensions than in RE, most likely due to the release of inhibitory compounds from vacuoles and other cellular compartments.

Fig. 5. Root penetration zones of *Pratylenchus thornei* in *Triticum aestivum*. **A to C**, Several *P. thornei* at lateral root branches. **D**, Groups of *P. thornei* probing and penetrating lateral roots. **E**, Several *P. thornei* at zone of elongation. **F**, Single *P. thornei* in the dense root hair zone. **G**, Aggregations of *P. thornei* juvenile and adult developmental stages within root regions.

The effective RE and CR solutions were derived from seedlings not exposed to *Pratylenchus* spp. or other plant pathogens, which implies the presence of constitutively produced compounds in resistant roots that can suppress *P. thornei* motility. In the nematode *Radopholus similis*, temporary nonmotility was observed after exposure to RE from both susceptible and resistant banana cultivars that had not been infested with the nematode. In all, $\leq 50\%$ were nonmotile after exposure to exudates from susceptible plants and the effects persisted for 1 to 3 days but $>80\%$ of nematodes were nonmotile for >6 days in the case of exudates from resistant roots (76). The observed inhibitory effect on nematode motility of the resistant root compounds was partially reversible because 50% of the nonmotile *P. thornei* recovered motility when washed with water up to 2 days after exposure. A similar reversible effect within 2 days was described for *P. scribneri* exposed to coumestrol. However, after 4 days, few motile nematodes had recovered after washing (48). Based on the current results, the compounds involved in *P. thornei* motility suppression appear to be nematostatic but not nematocidal and affect *P. thornei* but not *P. neglectus*. Resistant genotypes caused inhibition of motility of 50 to 75%; therefore, the role of these toxic compounds as defense mechanisms is significant. The motility assays designed in this study may provide useful alternative phenotypic resistance screening protocols.

Dual resistance to both *P. thornei* and *P. neglectus* is desirable because they commonly occur in wheat fields together (60). Generally, the investigated wheat sources of *Pratylenchus* resistance (AUS5205, AUS11984, and GS50a) do not contain resistance to both species at the same locus (15,59). However, resistance QTL to both *Pratylenchus* spp. from the same wheat source (CPI133872) have been identified to co-locate (81), suggesting that, in some cases, resistance mechanisms to both species may share the same biological mechanism. The specificity of the suppressive root compounds identified in this study was assessed by performing motility assays with both CR suspensions and RE on *P. neglectus*. Results showed low motility suppression (25%) in both resistant and susceptible roots. Although some loss in motility was observed, the proportion between the samples and the no-root water control was not significantly different. This

TABLE 3. Motility of *Pratylenchus thornei* 1 day after inoculation in different concentrations of root exudates collected from resistant (Sokoll) and susceptible (Krichauff) *Triticum aestivum* genotypes

Root exudate (mg/ml)	Sokoll		Krichauff	
	Nonmotile (%)	Standard error	Nonmotile (%)	Standard error
100	46.6	4.8	32.6	7.9
4	35.8	1.4	16.3	4.7
2	25.8	2.0	22.4	2.6

suggests that motility suppression caused by resistant root compounds investigated in this study is specific to *P. thornei*. Similar species specificity was observed with the inhibitory effect of coumestrol on *P. scribneri* but not on *M. javanica*, even at high concentrations (25 $\mu\text{g/ml}$) (48). Interestingly, the Sokoll $\times$ Krichauff population contains *P. neglectus* resistance, with Krichauff as the resistance source carrying the *Rln1* resistance gene (K. Linsell, unpublished data). However, as shown in this investigation, this resistance does not appear to be associated with motility suppression or is possibly not constitutively expressed.

Because it was established that resistant roots were able to suppress nematode motility in vitro, an experiment was designed to measure nematode migration in roots or, essentially, feeding within roots. The design meant that only a portion of one root was available for penetration, while the rest of the seedling's root

Fig. 7. Motility of *Pratylenchus thornei* in root exudates after 3 days of exposure from resistant (Sokoll, R1, and R2) and susceptible (Krichauff, S1, and S2) *Triticum aestivum* genotypes. LSD = least significant difference.

Fig. 8. Migration of *Pratylenchus thornei* within *Triticum aestivum* roots from the point of root inoculation, 10 days after inoculation in resistant (Sokoll, R1, R2, and R3) and susceptible (Krichauff, S1, S2, and S3) root genotypes. LSD = least significant difference.

Fig. 6. Motility of *Pratylenchus thornei* in **A**, root exudates and **B**, crushed root suspensions from *Triticum aestivum* after exposure for 3 days. Genotypes assayed were resistant (Sokoll, R1, R2, and R3) and susceptible (Krichauff, S1, S2, and S3). LSD = least significant difference.

system was physically blocked from the inoculated nematodes. These migration assays revealed that *P. thornei* did not migrate far through the root cortex from the point of inoculation in resistant roots. On average, nematodes were able to move >60 mm further in susceptible Krichauff than the resistant Sokoll. Because >50% penetration occurs within 9 hai on agar, root growth after inoculation will not affect the migration measured. It was essential that motility and migration assays were performed under sterile conditions because fungi are known to be sources of anti-nematodal compounds that can suppress motility and hatching (66,72).

Reproduction and maturation. The developmental stages of *P. thornei* collected from inoculated roots at multiple times across

45 days matched well to the life-cycle observations made by Larson (33), the only other published report of *P. thornei* reproduction in wheat. Therefore, the development timeline reported in this study under glasshouse conditions can now be utilized in future studies of specific *P. thornei* reproductive stages. The analysis of development over time between the resistant and susceptible genotypes within the Sokoll $\times$ Krichauff population showed significantly less *P. thornei* at all stages in the resistant compared with the susceptible roots. Similarly, reduced reproduction was observed in a resistant durum wheat cultivar, where no difference in penetration between the susceptible and resistant cultivars also occurred (58). Reduced reproduction indicates that either the nematodes exit resistant roots or fail to develop and

Fig. 9. Number (log transformed) of *Pratylenchus thornei* developmental stages within resistant (Sokoll, R1, R2, and R3) and susceptible (Krichauff, S1, S2, and S3) *Triticum aestivum* roots at different times within one nematode life cycle (45 days). **A**, Second-generation stage two juveniles (J2s) hatch at 36 days after inoculation (dai). **B**, Stage-three juveniles (J3s) first-generation molt at 10 dai and second generation at 45 dai. **C**, Stage-four juveniles (J4s) and adults first-generation molt at 14 to 28 dai. LSD = least significant difference.

reproduce. This study showed that *P. thornei* nematodes do not exit roots because large numbers are still present in the roots even after 16 days, indicating that reproductive development is suppressed. In the second generation, up to 10 times as many J2s and J3s were detected in susceptible roots as in resistant roots, suggesting that molting is also suppressed or delayed in the resistant genotypes. This is supported by Farsi (14), who observed a delay in molting of *P. neglectus* in resistant wheat cultivars, with only 1.7% molting, compared with 30% in the susceptible. Delayed maturation was also observed in *P. goodeyi* in resistant banana roots. Adult and J4 development were delayed by 4 days in comparison with the susceptible cultivar, which affected optimum egg deposition and hatch (45). The current study shows that

resistance expressed in the Sokoll $\times$ Krichauff population to *P. thornei* prevents juvenile maturation and, thus, reproduction.

Egg deposition and egg hatch. In addition to delayed maturation, experiments were conducted to determine whether egg deposition and hatching were also affected by the presence of resistant roots. *Pratylenchus* spp. deposit single eggs either in soil or within roots (40); both were examined in this study. Adult *P. thornei* did deposit eggs within and near resistant roots but egg deposition was up to 30 times less than in susceptible roots. Farsi (14) reported similar suppression for *P. neglectus* in resistant wheat cultivars, with egg deposition being delayed by 4 days compared with the susceptible cultivar and no eggs being observed in the investigated resistant cultivar even after 35 days.

There is limited data on the capacity of *Pratylenchus* spp. to deposit eggs (25). However, the maximum egg deposition rate of *P. penetrans* and *P. brachyurus* was 2.7 to 4.8 eggs/day (35), both over 11 days at 20°C. In the current study, mean deposition rates for *P. thornei* were 4.7 eggs/day over 10 days at 21°C in Krichauff. This suggests that temperature, plant, and nematode species all affect the rate of egg deposition. The rate of egg deposition per female per day in the susceptible roots was almost three times higher than in the resistant roots. Thus, resistance appears to decrease the rate of egg deposition of *P. thornei* in the investigated wheat genotypes. In addition, fewer eggs were laid on agar plates where no roots were present, suggesting that *Pratylenchus* nematodes deposit eggs only when a food source is available. In susceptible plants, females preferentially deposited eggs within the roots (83%) rather than outside. Only half the numbers of eggs were deposited within and adjacent to the resistant roots. A lower deposition of eggs within resistant roots

Fig. 10. Egg deposition of *Pratylenchus thornei* in and near *Triticum aestivum* seedling roots propagated on agar. **A**, Number of eggs deposited in (roots) and directly adjacent (agar) to resistant (Sokoll, R1, and R2) and susceptible (Krichauff, S1, and S2) root genotypes. **B**, Rate of eggs deposited per day in the resistant and susceptible genotypes. LSD = least significant difference.

Fig. 12. Number of *Pratylenchus thornei* juveniles hatched from eggs soaked in serial dilutions of root exudates from resistant (Sokoll, R1, and R2) and susceptible (Krichauff, S1, and S2) *Triticum aestivum* genotypes. LSD = least significant difference.

Fig. 11. Hatching of *Pratylenchus thornei* near *Triticum aestivum* seedling roots propagated **A**, on agar and **B**, within root exudate solutions. **A**, Number of juveniles hatched within (roots) and directly near (agar) resistant (Sokoll) and susceptible (Krichauff) roots. **B**, Number of juveniles that hatched in resistant (Sokoll, R1, and R2) and susceptible (Krichauff, S1, and S2) root exudates after exposure for 3 days. LSD = least significant difference.

may be due to a higher concentration of suppressive compounds or could be due to the reduced migration and feeding, so that mature females fail to acquire the energy to produce eggs. Reduced egg deposition has been associated with *P. goodeyi* resistance in banana where, in the susceptible roots, *P. goodeyi* nematodes deposited 29.8 eggs/day compared with 18.8 eggs/day in the resistant roots (45).

Hatching is a complex biological process affected by environmental factors and, sometimes, by the host (54,55). In general, nematode hatching is significantly influenced by temperature, soil type, and moisture (13,17,50,57). REs can contain hatching factors (HFs) that may stimulate hatching in some species of nematodes; and HIs, which prevent juvenile emergence from eggs. It is known that most species of nematodes do not solely rely on host-derived stimuli to initiate hatching and that many hatch freely in water (43), including *P. thornei*, as observed in this study. However, host signals are vital to ensure that significant numbers of juveniles emerge within a close vicinity of the roots to increase rates of successful penetration (43) or to induce hatch after a period of nematode dormancy (26). Byrne et al. (8) showed that hatch in response to potato root leachate of *Globodera rostochiensis* on agar correlated well with hatch in soil. Thus, hatching in this study was performed in vitro both on agar and in RE.

Significantly fewer *P. thornei* juveniles hatched from eggs within and adjacent to the roots of resistant versus susceptible seedlings grown on agar. Hatching was also decreased in resistant RE compared with susceptible but only by 8% after 10 days of exposure. The smaller hatching suppression observed in resistant RE compared with actively growing resistant roots on agar could be due to the presence of less HI in the 4 mg/ml concentration of RE used than that naturally exuded by live roots. In addition, it could be that the active compounds in RE are labile and become inactive over time (10 days). In the susceptible roots and in the no-root control, only 50% of *P. thornei* juveniles had emerged from deposited eggs after 10 days. Similarly, a maximum of 50% *P. penetrans* hatched even though 64% of the eggs already contained J1s or J2s during in vitro tests under optimum conditions (47). This suggests that despite uninterrupted juvenile maturation and deposition of eggs in favorable environmental conditions, not all juveniles will hatch. Similar suppression of hatching by resistant REs has been observed in other plant/nematode interactions. RE from susceptible soybean cultivars stimulated more hatch and emergence of *H. glycines* than resistant cultivars (9,54,55). Similarly, partially resistant clones of *Solanum* spp. hybrids generally stimulated lower hatch of *G. pallida* than susceptible cultivars after several weeks of exposure to RE (16). The flavonoids glyceollin and coumestrol, which suppressed *H. glycines* and *P. scribneri* motility as discussed earlier, also significantly affect egg hatch (64). Flavonoids or their derivatives such as ferulic, syringic, and coumeric acid are known to be present in wheat roots (21,22,34,53) and could explain the hatching suppression in resistant REs observed in this investigation.

The observed resistance at hatching could be explained by a loss of an HF or the presence of HI. In addition to HF, there are HF stimulants, which are compounds that are hatch-neutral but can enhance the function of HF (8). The observation that a lower hatch rate occurred in resistant roots compared with the no-root control indicates the presence of HIs. The presence of HIs was further shown through dilution of the RE. Because HIs occur in low concentrations and HFs in much higher, dilution of RE should predominantly affect HIs without removing the activity of HFs (8). As the RE became more diluted, there was an increase in hatching in the resistant roots, thus further suggesting the presence of HIs for *P. thornei*. The observed decrease of hatching associated with the resistant wheat lines may be explained by inhibition or delay of egg hatch.

In conclusion, in the Sokoll × Krichauff population investigated, resistance acts post penetration with the suppression of migration, juvenile maturation, and, thus, reproduction. Consistent with various other plant-parasitic nematode species, there was no significant difference in the rate at which *P. thornei* was attracted toward or penetrated resistant or susceptible roots. No preferential root penetration zone was observed, unlike in other studies; however, once inside the root, groups of *P. thornei* at various developmental stages moved in the tracks left by previously migrating nematodes. Migration and motility of *P. thornei* is suppressed in resistant roots and exudates, suggesting that resistant genotypes constitutively produce compounds that inhibit motility. The effects of these compounds are reversible and differentially affect *P. thornei* but not *P. neglectus*.

Nematode multiplication was inhibited by resistance because juvenile maturation was suppressed in resistant roots, with very few developing past J3. *P. thornei* J2s were still present within the roots 10 dai; therefore, juvenile maturation is inhibited by resistance rather than causing the nematodes to exit resistant roots. In addition, egg deposition and hatch of *P. thornei* was significantly reduced in resistant roots and exudates. The increased hatch after dilution of RE and the lower hatch in resistant exudates versus the absence of roots indicates the presence of HIs. The actions of flavonoids and phenolics have been linked to *Pratylenchus* motility suppression in other resistance responses and, thus, are targets for future investigations into the resistance of *P. thornei* to Sokoll × Krichauff mapping population lines. Further characterization of biological resistance mechanisms and the development of robust screening protocols will enhance the development of *P. thornei* resistance breeding in wheat.

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